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JOSIAH MACY, JR. FOUNDATION
One Rockefeller Plaza, New York, N.Y. 10020

Faculty Scholar Award Program

Confidential Reference Report

Applicant's Name Morton Mandel

Name of Reference J. Lederberg

Title and Institution Professor, The Rockefeller University

Address Office of the President, New York, N. Y. 10021

The above applicant for a Macy Faculty Scholar Award has named you as one of several references. Enclosed is the applicant's statement of the project he wishes to undertake. We ask your cooperation in commenting on the applicant's statement of plans and the suitability of the institution at which he proposes to carry them out. In addition, we would appreciate a brief statement of the nature and length of your relationship with the applicant and your overall appraisal of the candidate as an individual and as a medical scientist.

Your response will be held in strict confidence. PLEASE FORWARD YOUR COMMENTS DIRECTLY TO THE FOUNDATION BY OCTOBER 24.

The Macy Faculty Scholar Awards are designed to assist outstanding faculty members of schools of medicine and public health in continuing their professional growth by taking sabbatical leaves. The awards are for a maximum period of one year and a minimum period of six months.

(Please use both sides of this form and additional sheets if necessary for your reply.)

Date____________________

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Signature of Reference

Using metaphase chromosome-mediated gene transfer, a unique line of cultured mouse cells have been developed. The technique requires two closely related mouse cell lines and employs as the vector a cell-free mixture of metaphase chromosomes and nuclei prepared from cells carrying hprt, the gene encoding for hypoxanthine-guanine phosphoribosyl transferase (HPRTase). Mutant cells containing no detectable HPRTase activity were used as recipients and the donor cell carried an altered HPRTase so as to be able to distinguish between cell transferents and revertants. This gene transfer process is characterized by (a) a low frequency of transferent clones, (b) the very small size of the genetic fragment transferred and (c) an initial instability of the transferred gene. In the absence of selection these gene-transferent cell lines have about a 10% chance of losing the hprt gene at each cell division. Stabilized subclones can be isolated from these unstable lines.

The key observation pertinent to this research proposal is that in the initial unstably inherited state, the hprt gene is greatly overexpressed, resulting in HPRTase activity 20- to 60-fold higher than in the donor parental strain. When stabilized clones are isolated (once in 100,000 cell divisions) they are found to return to the donor level of HPRTase activity. Recent work in Dr. Eisenstadt's laboratory has shown that this unstable gene is most likely carried in the nucleus of the cell (determined by karyoplast-cytoplast fused cells). These observations suggest (1) an autonomously replicating mammalian genetic unit (a single gene, hprt, or a small group of linked genes) has been produced in the host nucleus, possibly a circular DNA molecule, (2) for reasons which are not now understood but which must relate ultimately to its non-chromosomal location, the hprt genes over-produce its enzyme. If a single gene copy is present the phenomenon is a "positive effect" and reflects absence of regulatory factors. If multiple copies are present the phenomena is a "gene dosage effect", (3) the gene is often lost from the daughter nucleii at mitosis since it is not linked to a spindle attachment site, (4) integration of the gene into a host cell chromosome stabilizes the hprt gene inheritance and restores normal gene activity.

Two aspects of these cell lines appear to be important model systems, the ability of a small piece of mammalian nuclear DNA to replicate autonomously and the apparent escape from normal cellular control mechanisms of a gene carried in this way. This genetic fragment may, therefore, provide the opportunity to study in a meaningful way the control of gene replication and the regulation of gene activity.

The specific aims of this research proposal are (1) to determine the location (extra-chromosomal or chromosomal) of both the stably and unstably inherited gene, (2) to determine the number of the gene copies in the unstable cell (one copy of 20-50 copies), (3) to characterize the DNA which contains the hprt gene and (4) to isolate from unstable cells the specific DNA fragment which contains the hprt gene. All of the above work will depend on the synthesis of a DNA probe complementary to the hprt gene. The high rate of HPRTase enzyme activity in the unstable cell line has changed the proportion of enzyme protein from 0.02% to about 0.5 to 1%. Such an elevated level should permit the isolation and purification of hprt messenger RNA (mRNA) using an immunological approach based on the specificity of an antibody prepared against the native protein and reacting with nascent peptide chains associated with polysomes. This technique has been used to separate ovalbumin mRNA from all other cellular mRNA's.

Using purified homogeneous hprt-mRNA containing poly-A3' ends as a template, a highly radioactive complementary DNA (cDNA) probe will by synthesized by avian myeloblastosis virus reverse transcriptase employing oligo-dT priming fragments. The radioactive probe thus generated will be hybridized to total unlabeled DNA prepared from the appropriate mouse L. cell strains. The degree of hybridization at various times will be assayed by conversion of the input radioactivity to S-1 nuclease-resistant acid-precipitability using hydroxyapatite columns to select hybrid DNA (Cot curves).

The kinetics with which input cDNA forms hybrids with excess total DNA is a sensitive measure of the number of gnee copies per genome present in the tested DNA and has served to discriminate between one and two to three copies per haploid genome as in the case of SV 40 transformed cells. The discrimination we need (one copy vs 20 to 60) is well within sensitivty of the method. Throughout these experiments the genetic stability and enzymatic levels of the strains being used will be monitored to insure against accidentally selecting stabilized variants.

Using total DNA from transferents and the cDNA probe for the hprt gene two experiments can be done to characterize the DNA which contains the gene. The molecular weight of the hprt sequence can be determined by gently lysing transferents on alkaline sucrose gradients (conditions which cause very little DNA breakage) and hybridizing across the gradient with the cDNA probe. This technique has been used to show that SV 40 DNA is covalently integrated into the DNA of transformed cells. The linkage of hprt sequences to highly repetitive DNA sequences can also be determined as follows. DNA from transferents sheared to appropriate size, will be thermally denatured and then self-annealed just long enough that repeated, but not unique, sequences reassociate.

Under these conditions, large networks of DNA form and can be isolated by sedimentation (or hydroxyapatite isolation of the hybridized DNA can be used). Finding most or all hprt sequences in the pellet upon rehybridization of gradient fractions with cDNA probe indicates covalent linkage between hprt and repetitive DNA. Each of these experiments will provide information about the molecular state of hprt gene in both stable and unstable transferents.

A general technique to be used to isolate the DNA fragments containing the hprt gene and possible adjacent sequences is as follows: hprt-specific mRNA would be transcribed with reverse transcriptase to form cDNA. The cDNA would be mercurated in either of two ways, either using mercurated nucletoide triphosphate as substrates during cDNA synthesis or by direct covalent mercuration of the polynucleotide by incubating the cDNA at 37-50° in buffered aqueous solution of mercuric acetate. The hprt-specific mRNA can also be directly mercurated and used as probe. Themercurated probe (either mRNA or cDNA) would be hybridized to heat denatured DNA isolated from the unstable cell line. The hybrid molecules are then passed through a sulfhydryl sepharose column under conditions where the mercurated molecules are bound. The column is washed to remove unbound nucleic acids and the hybrid is eluted with mercaptoethanol. The hybrid molecule can be denatured and the probe separated from the cellular DNA by a variety of methods.

The Department of Human Genetics of Yale is outstanding in both its basic and chemical work. Dr. Eisenstadt's laboratory in particular has made significant contributions to mammalian mitochondrial genetics, somatic cell genetics and chromosomal mediated gene transwer work and at the present time is the only laboratory with the unique cell line described above.